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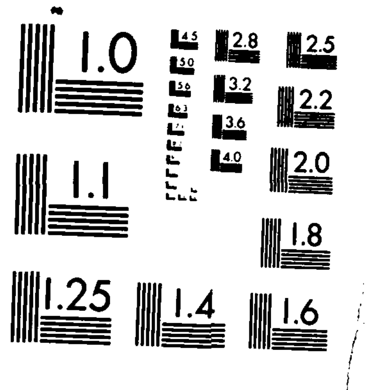

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HOST CELL SURFACE EXPRESSION OF RICKETTSIA TYPHI ANTIGENS DURING INFECTION

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Immune responses to rickettsial infection have been attributed to both humoral and cellular mechanisms. Although it has been shown that transfer of immune cells (1) or serum (2) can mediate resistance to challenge, the exact immune mechanisms involved remain poorly understood. Since rickettsiae are obligate intracellular bacteria, an immune attack upon rickettsiae while they are still inside the host cell would represent a powerful immunologic defense. In the present report, we show that tissue culture cells infected with Rickettsia typhi express rickettsia-specific antigens on their surfaces. Such antigens may provide triggering mechanisms for both the afferent and efferent arms of the immune system. Keywords: Endemic typhus,

MATERIALS AND METHODS

1. Cell line: The LS-929 cell line is a suspension variant of the fibroblast-like cell line derived from a C3H/AnN mouse. It is maintained in Eagle's MEM supplemented with L-glutamine and fetal calf serum, without added antibiotics.

2. Rickettsia: Rickettsia typhi strain Wilmington was grown in the yolk sac of embryonated chicken eggs, purified by isopycnic banding in Renografin 76 density gradients, and controlled-rate frozen to -100°C at 0.5 mg protein/0.5 ml in Bovarnick's sucrose phosphate glutamate supplemented with 5 mM $MgCl_2$ and 1% Renografin 76.

3. Fluoresceinated reagents: Rabbit antiserum to R. typhi (RbαRt) was raised by multiple injections of intact and disrupted purified rickettsia in Freund's incomplete adjuvant. Antibodies were purified by salt precipitation and fluoresceinated by standard methods. As a negative control, rabbit anti-yolk sac (RbαYS) antibodies were raised, purified and fluoresceinated in the same way as RbαRt.

4. Flow cytometry: Fluorescence analysis was carried out on a FACSII (Becton, Dickinson, Sunnyvale, CA) equipped as described elsewhere (3,4). For surface analysis, the cells to be tested were washed twice in HBSS containing 0.01% NaN_3. The cell pellet was resuspended and stained with fluoresceinated reagents prepared as described above. After 45 min on ice, the cells were washed again and fixed in 1% paraformaldehyde.

RESULTS

In Fig. 1 it can be seen that a subpopulation of LS-929 cells infected with R. typhi 72 hours earlier expresses surface antigens that are detectable with RbαRt antiserum. That this reaction is not caused by nonspecific stickiness of the infected cells was shown by the negative staining profile of RbαYS tested on R. typhi infected LS cells which completely overlapped the background fluorescence profile given by unstained infected or uninfected LS-929 (not shown). Dead cells are excluded from the analysis by forward light scatter. When the kinetics of acquisition of this surface antigen were examined it was found that as early as 24 h after infection, a shoulder of positively staining cells appeared. These positive cells were increased in number on day 2, reaching a maximum on day 3.

As noted in Fig. 1, only a subpopulation of infected cells bears the antigen on its surface. Even though the entire population was exposed to R. typhi organisms, it is possible that only some LS-929 cells became infected, thereby giving the biphasic pattern seen. However, by light

microscopy on day 3 all the cells infected with R. typhi contained numerous rickettsiae, although only 42% expressed surface antigens, as shown in Fig. 1. The possibility that expression of rickettsial antigens on the cell surface is dependent on cell cycle is under investigation.

Since R. typhi infected cells may liberate intact organisms, or soluble antigens into the culture medium, it is possible that this antigen may passively adsorb on the surfaces of cells. Four different approaches were used in this study.

In the first approach, LS929 cells were infected at a high multiplicity, and stained one hour after infection. This experiment (not shown) examined the possibility that rickettsiae were leaving antigen on the cell surface upon penetration. No staining was observed.

In the second approach, uninfected LS-929 cells were incubated with sonicated whole rickettsiae for three days at 37°C. The cells were then washed and stained with antiserum as described above. The results of a representative experiment are shown in Fig. 2A. At the highest doses of sonicate used (4 ug/flask) the FACS pattern virtually overlaps that of cells incubated with no antigen.

The third approach used was to measure the amount of rickettsial antigen in supernatant from infected cells. Using a sensitive enzyme-linked immunosorbent assay (ELISA) and an antigen-capture assay, titrated amounts of supernatant from three-day infected LS-929 cells were analyzed for the amounts of antigen they contained. The results of these experiments, suggested that only small amounts of antigen were present: about 100-300 ng/ml of supernatant. This range is marked with an asterisk on Fig. 2A.

The fourth approach involved transfer of culture supernatant from three day infected LS-929 to uninfected cells, incubating an additional three days, and then examining the cells for their ability to bind RbxR.t. antiserum. Figure 2B shows the results of such an

experiment. It can be seen that LS-929 cells incubated for three days with culture supernatant from infected cells (containing 100-300 ng/ml antigen) did not exhibit the binding pattern seen after a three day infection (as shown in Fig. 1). These experiments strongly suggest that the appearance of rickettsial antigens on the surface of infected cells is dependent upon an ongoing infection, and is not due to external adsorption of antigen from the culture supernatant.

DISCUSSION

In theory organisms which replicate intracellularly and migrate from cell to cell might be relatively sequestered from the systemic effects of antibodies, or the local effects of cellular immune mechanisms. Further, intracellular organisms may not be presented as immunogens in an efficient manner. In the present report, we show that L-cells infected with the obligate intracellular bacterium, Rickettsia typhi express antigens on their surface which are recognized by an antibody directed to rickettsiae.

It is known that cells infected with other intracellular organisms such as viruses (5) protozoa (6) or bacteria (7) express antigen on their surface. In contrast to viral antigens, which are expressed on the infected cell surface within hours (7), the rickettsial antigens required a few days for maximum expression. This may reflect the slower replication time of rickettsiae relative to viruses. Since cycloheximide inhibits expression of rickettsial antigen under these conditions (Rollwagen and Dasch, paper in preparation), it might be inferred that a eukaryotic host processing (glycosylation?) step is involved. These mechanisms are currently under study in our laboratory.

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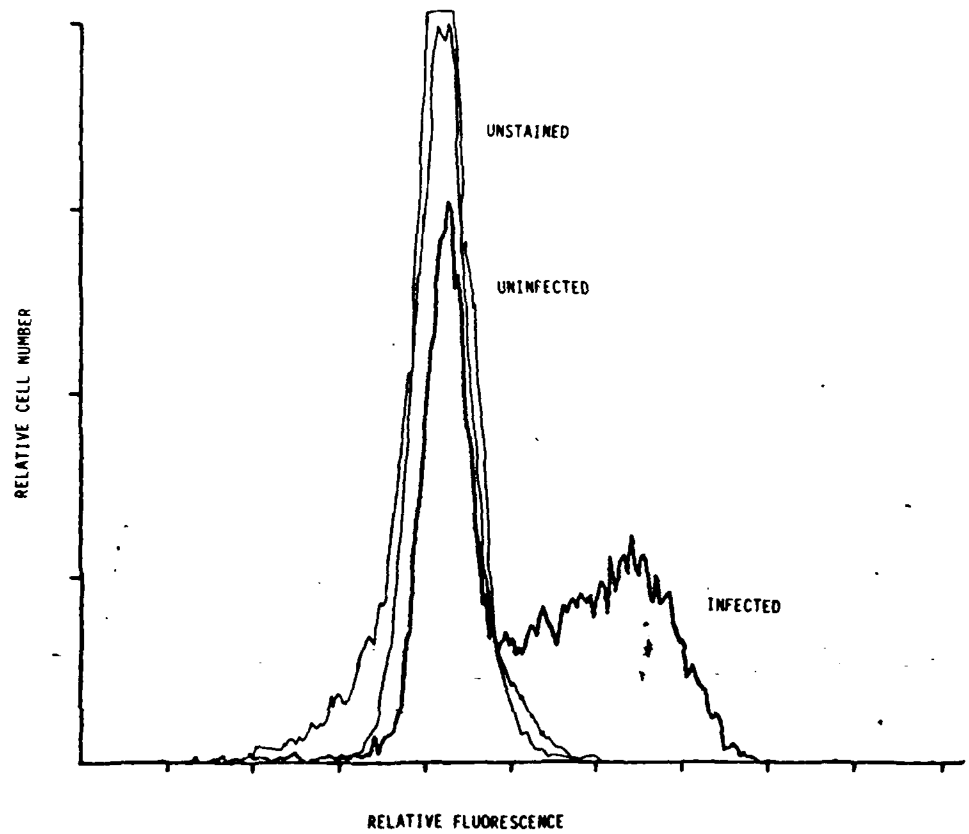

Figure 1. FACS profile of LS-929 cells uninfected or infected with R. typhi stained with fluoresceinated antibody to R. typhi or unstained.

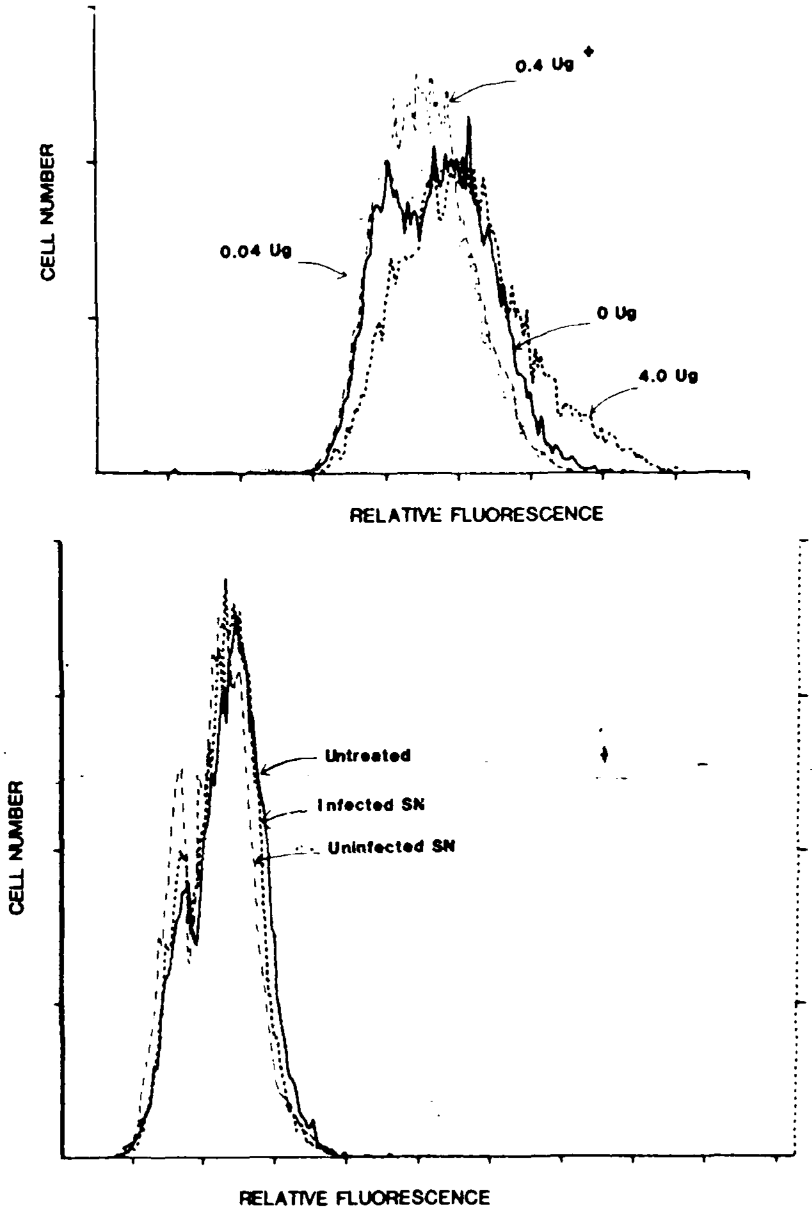

Figure 2. A. Uninfected LS-929 cells incubated with sonicated whole rickettsiae for 3 days <u>in vitro</u>, washed and stained with RbαRt

B. Supernatant from 72-hour infected or uninfected LS-929 was transerred (1:1) to uninfected LS-929 and incubated for 3 days. The cells were washed and stained with RbαRt

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